A Simulation and Estimation Platform for Malaria Model Evaluation

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ABSTRACT

BACKGROUND: Accelerating clinical development of new compounds demands efficient systems for evaluation and interpretation of trial results. Systematizing trial evaluation methods yields efficiency and confidence in results. A simulation/estimation (S/E) platform was employed for definitive assessment of parasite models used for analysis of volunteer infection studies (VIS). Using rich data, parasite models were evaluated for identifiability and performance.

METHODS: Simulated hourly parasite counts (mrgsolve; 500 replications) were analyzed (NONMEM 7.3; KIWI 2) with 4 structural models with various random effects (RE). Three empirical models (traditional first-order growth and drug effect [TFGDE], indirect response [IDR], and Gompertz [GOMP]) and a semi-mechanistic model (Gordi) were evaluated. Recrudescence, limit of quantification (LOQ of 10 or 111 parasites/mL), growth phase, and drug effects were considered.

RESULTS: The TFGDE with RE on 2 and 5 parameters and Gordi with RE on 3 parameters were most stable with respect to identifiability and precision of parameter estimates. For TFGDE and Gordi models with LOQ = 10, drug effect was well estimated with EC_{50} (0.0123 mcg/mL; 95% confidence interval [CI] = 0.0122 - 0.0123) and Kpinj (0.329 mcg/mL x h; 95% CI = 0.328 - 0.331), respectively.

CONCLUSION: Traditional and Gordi models perform well. Further work on LOQ and limited data scenarios is needed. The S/E platform allows assessment of relative model performance to guide efficient model selection and refinement.

INTRODUCTION

- The parasite lifecycle (Fig. 1) is complex; parasites traverse through the liver and undergo a cyclic asexual replication in the blood. Parasitized erythrocytes are cleared from the human host through various host defense mechanisms [1].
- VIS using the induced blood stage malaria (IBSM) model are a valuable system for defining the key pharmacokinetic/pharmacodynamic (PK/PD) relationships for dose selection in antimalarial drug development [2].
- Healthy volunteers are inoculated with a known quantity of *Plasmodium*-infected red cells. Parasitemia is measured by quantitative polymerase chain reaction (qPCR) until a prespecified treatment threshold is reached and the test drug is administered. Parasite and drug concentrations are measured throughout treatment.
- PK/PD modeling of data generated from IBSM studies provides the ability to predict and simulate drug concentrations and parasite counts to support clinical trial simulations and model-driven decision-making in antimalarial drug development.

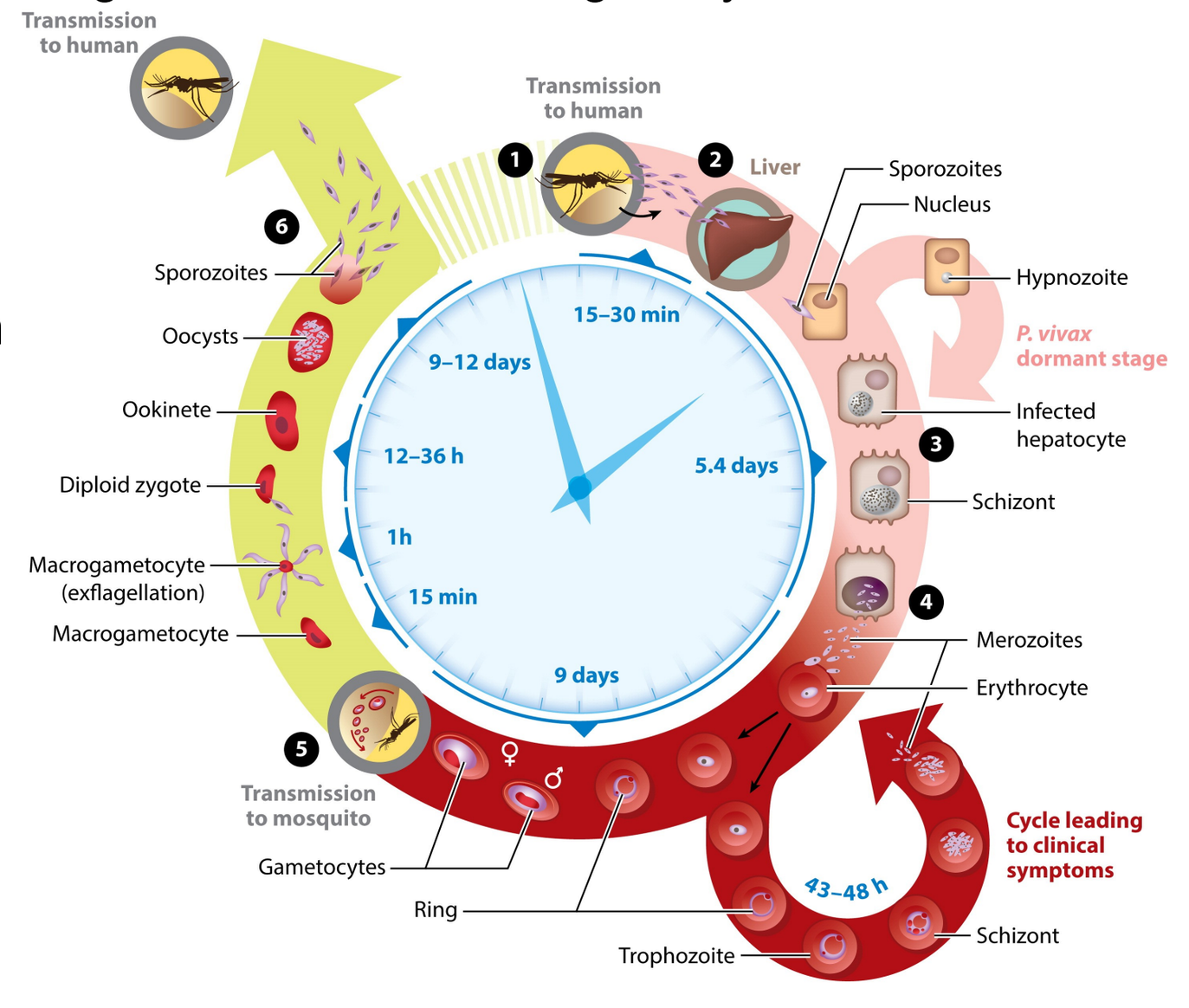
Transmission to human
Transmission to human
Liver
Sporozoites
Nucleus
Hypnozoite
P. vivax dormant stage
Infected hepatocyte
Schizont
Merozoites
Erythrocyte
Cycle leading to clinical symptoms
Schizont
Trophozoite
Ring
Gametocytes
Transmission to mosquito
Macrogametocyte
Macrogametocyte (exflagellation)
Diploid zygote
Ookinete
Oocysts
Sporozoites
15–30 min
9–12 days
12–36 h
5.4 days
1h
15 min
9 days
43–48 h
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Figure 1. Parasite lifecycle

RATIONALE

- As drug discovery methods become more advanced and target biomarkers on the parasite become more readily available, increasingly more mechanistic pharmacodynamic (PD) models can be used to model the IBSM data.
- Currently, a linear growth function is typically used to characterize net parasite growth and a Hill function is used to represent drug-induced parasite death [3,4,5]. However, alternative PD models have been fit to IBSM data (unpublished work).
- The use of non-identifiable models may cause numerical problems during estimation and yield unreliable, imprecise parameter estimates that are not informative for decision-making.
- Prior to this work, a formal evaluation of the pharmacostatistical models combining data prior to and post antimalarial dose in IBSM studies had not been published.

GOALS and OBJECTIVES

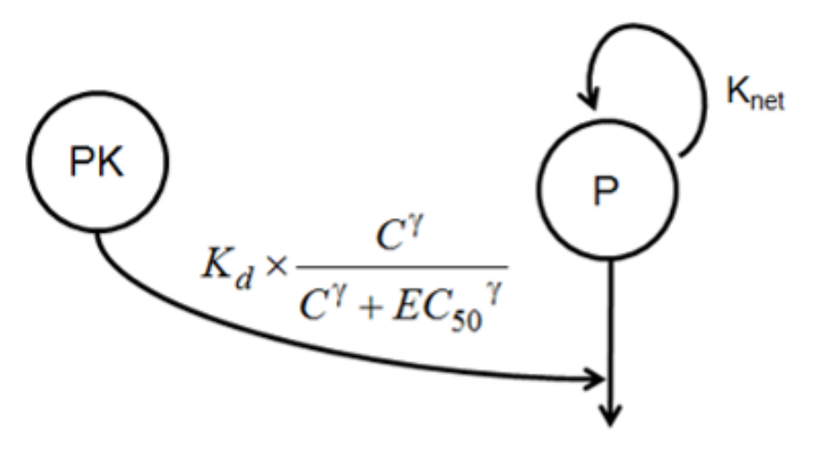
- The **goals** of this analysis were to
  1. understand the potential for bias of parameter estimates, and issues with parameter identifiability and precision, for each tested model and
  2. provide a scientific basis for model selection and refinement for analysis of IBSM study data.
- The **objectives** of this work were to:
  1. simulate rich datasets from each of the 4 empirical candidate models and
  2. for each of the models, estimate the population PD parameters and their variability to give insight into model identifiability.

METHODS

Models

- Four models were evaluated. Three of the 4 models are variations on the traditional maximum pharmacologic effect (E_{max}) model; the fourth (Gordi, *et. al*) was a semi-mechanistic model.
- The E_{max} model with linear growth (Eq. 1) has been widely used in the literature to represent IBSM data and assumes a net growth of parasite, collapsing the growth rate and natural death rate into 1 parameter, K_{net} [3,4,5].

Traditional Model (TFGDE)



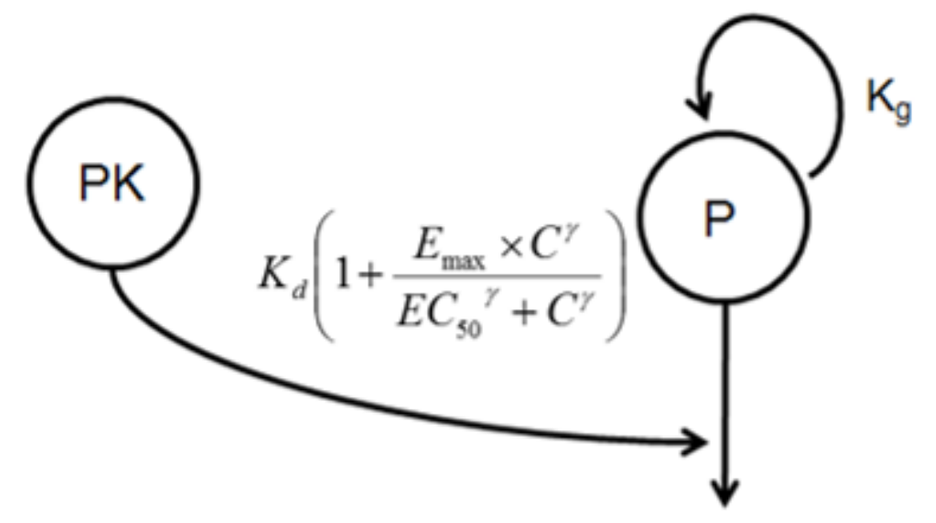
$$\frac{dP}{dt} = P \times \left(K_{net} - K_d \times \frac{C^{\gamma}}{C^{\gamma} + EC_{50}{}^{\gamma}} \right)$$

Equation 1

Where:
- P is parasite count;
- K_d is maximum first-order rate constant for drug-induced death of parasite (1/h);
- γ is Hill coefficient;
- EC_{50} is drug concentration at which 50% of maximum rate of parasite death occurs (µg/mL); and
- K_{net} is first-order rate constant for net growth of parasite (1/h).

Indirect Response Component Model (IDR)

- In an effort to conceptually separate the natural growth and death rates of parasites, the traditional model shown in Eq. 1 was adapted to include an indirect response component, as shown in Eq. 2 (the "IDR" model).
- This model has a first-order input of parasite growth and drug effect stimulates the loss of parasites from the system [6].
- Maximum drug effect is a fold increase above natural parasite death through the E_{max} parameter.



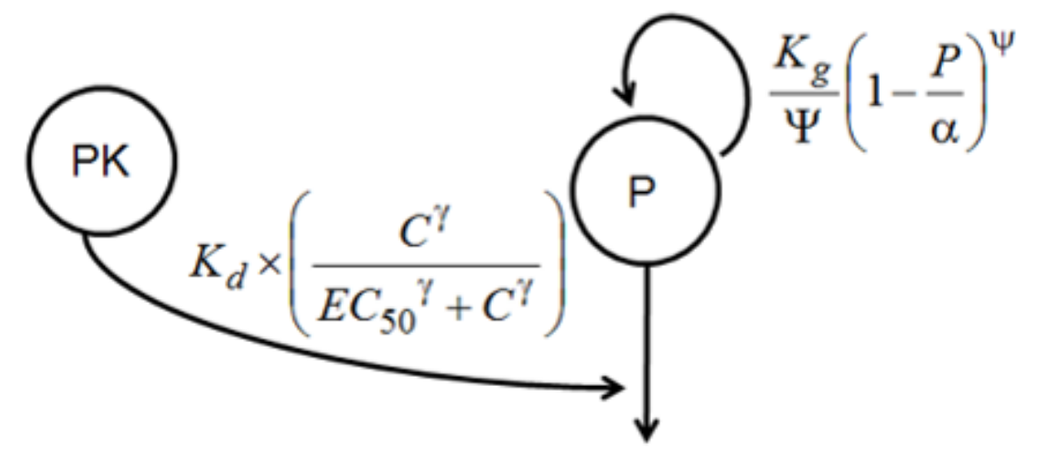
$$\frac{dP}{dt} = P \times \left(\left(K_g - K_d \left(1 + \frac{E_{max} \times C^{\gamma}}{EC_{50}{}^{\gamma} + C^{\gamma}} \right) \right) \right)$$

Equation 2

Where:
- P is parasite count;
- γ is Hill coefficient;
- K_g is first-order rate constant for growth of parasite (1/h);
- K_d is first-order rate constant for natural death of parasite (1/h);
- EC_{50} is drug concentration at which 50% of maximum rate of parasite death occurs (µg/mL); and
- E_{max} is fold increase of drug-induced death above K_d (unitless).

Gompertz Model (GOMP)

- Parasite growth was modeled using a Gompertz-type function in an effort to more accurately describe the nature of parasite growth.
- The growth of parasite is stunted by a deceleration value, Ψ, and limited by a maximum parasite count (α), and drug-induced death is represented with a Hill function (Eq. 3). K_d refers to the drug-induced death of parasite.



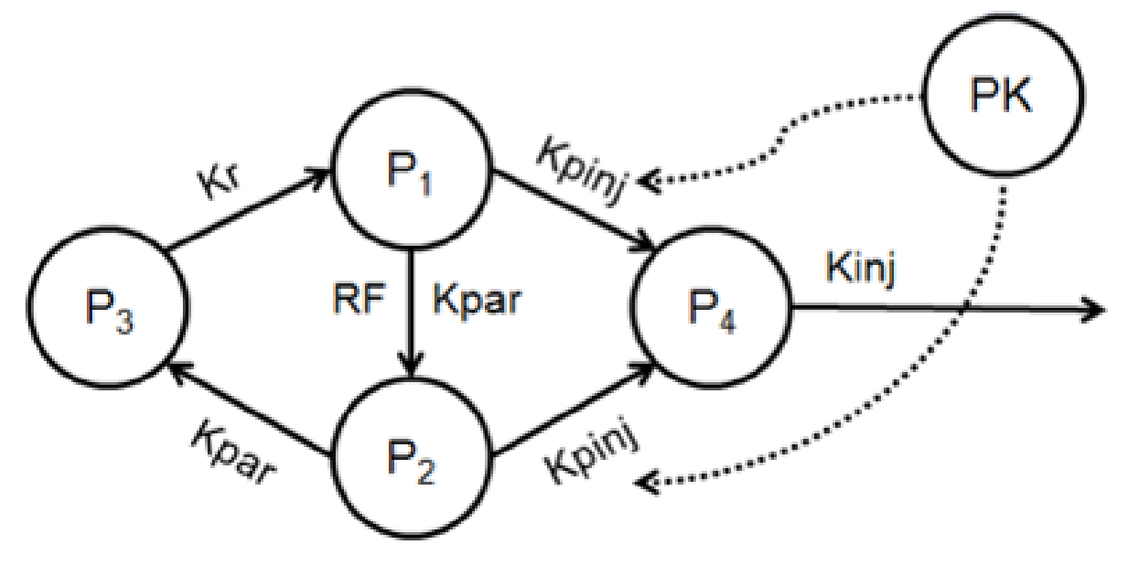
$$\frac{dP}{dt} = P \times \left(\frac{K_g}{\Psi} \left(1 - \frac{P}{\alpha} \right)^{\Psi} - K_d \times \left(\frac{C^{\gamma}}{EC_{50}{}^{\gamma} + C^{\gamma}} \right) \right)$$

Equation 3

Where:
- P is parasite count;
- K_g is maximum first-order rate constant for net growth of parasite (1/h);
- K_d is maximum first-order rate constant for drug-induced death of parasite (1/h);
- γ is Hill coefficient;
- EC_{50} is drug conc. at which 50% of maximum rate of parasite death occurs (µg/mL);
- α is asymptote for maximum parasite growth (parasite/mL); and
- Ψ is deceleration value.

Gordi Model (GORDI)

- A semi-mechanistic model (Eq. 4), the Gordi model differs from the other models evaluated in that this model includes 4 parasite compartments, representative of various stages of the asexual parasite lifecycle.
- The observed parasite count is a summation of 3 compartments (vpara = $P_1 + P_3 + P_4$), and compartment P_2 represents parasites that are sequestered or "invisible" from analytical detection [2].



Note: Mean transit time (MTT) of parasite was estimated in lieu of Kpar and Kr (h).
Where: Kpar = 2/MTT and Kr = 1/(48 - MTT)

Equation 4

$$\frac{dP_1}{dt} = P_3 \times Kr - P_1 \times Kpinj \times C - P_1 \times Kpar \qquad \frac{dP_2}{dt} = RF \times Kpar \times P_1 - P_2 \times Kpar - P_2 \times Kpinj \times C$$

$$\frac{dP_3}{dt} = -P_3 \times Kr + P_2 \times Kpar \qquad \frac{dP_4}{dt} = -P_4 \times Kinj + P_1 \times Kpinj \times C + P_2 \times Kpinj \times C$$

Where:
- P_1 is trophozoite (sensitive parasites);
- P_2 is sequestered schizont (sensitive parasites);
- P_3 is ring (insensitive parasites);
- P_4 is injured parasites;
- *vpara* is $P_1 + P_3 + P_4$; estimates the individual parasite count (dv);
- *RF* is trophozoite to schizont replication factor;
- *Kpar* is transit rate parameter from trophozoite to schizont to ring (h);
- *Kr* is transit rate parameter from ring to trophozoite (h);
- *Kpinj* is injury of trophozoite and schizont (µg/mL x h);
- *Kinj* is first-order removal of parasites by spleen (1/h).

Software

Simulations used the R package mrgsolve Version 0.8.10, R 3.4.2, and R Studio 1.1.383. PK/PD model estimation was performed in NONMEM Version 7.3.0, using KIWI™ Version 2.

Simulation and Estimation Methodology

- The overall workflow for the simulation and estimation process is shown in Fig. 2. The simulation study design and dosing regimens, hypothetical bioanalytical techniques, and lower limits of quantitation (LLOQs) are shown in Table 1a and Table 1b.

Table 1a. Simulation Study Design and Dosing Regimens

Study Title	Virtual Patients	Dosing Regimen	Sampling (PK/PD)
Simulation Study	250	Oral administration of 800 mg of quinine every 8 hours for 7 days, beginning 168 hours after inoculation	Every hour from 168 to 672 hours after inoculation

Table 1b. Simulation Study Bioanalytical Techniques and Lower Limit of Quantitation

Study	Pharmacokinetic		Pharmacodynamic	
	Endpoint and LLOQ	Assay / Method	Endpoint and LLOQ	Assay / Method
Simulation Study	Quinine plasma concentration; 1 µg/mL	Liquid chromatography with fluorometric detection	Total parasite; 111 parasites/mL and 10 parasites/mL	qPCR 18s

- The PK model was adapted from literature [7]. Hourly PK samples were simulated from 168 to 672 hours after inoculation.
- Simulation parameter values used for all models allowed for a similar range of parasites (1×10^3 - 1×10^5 parasites/mL) at Day 7 (168 hours) just prior to study drug administration [8].
- Cured was defined as parasite counts at or below 0.003 parasites/mL (threshold for cure).
- The time of rescue medication administration was imputed:
  - Evaluated samples every 8 hours after > 24 hours postdose.
  - Rescue medication is typically administered in an IBSM study when parasite counts return to ≥ 1×10^5 after an initial response (decrease in parasite counts) or in absence of a response 36 hours after inoculation.
  - The time at which a decrease in parasite count was determined. Then a search for the first parasite count ≥ 1×10^5 was performed. The time of this count was the imputed time of rescue medication.
  - If a decrease in parasite count was not found after 24 hours postdose, the imputed time of rescue medication was 36 hours postdose.
  - All samples after the imputed time of rescue medication were deleted.

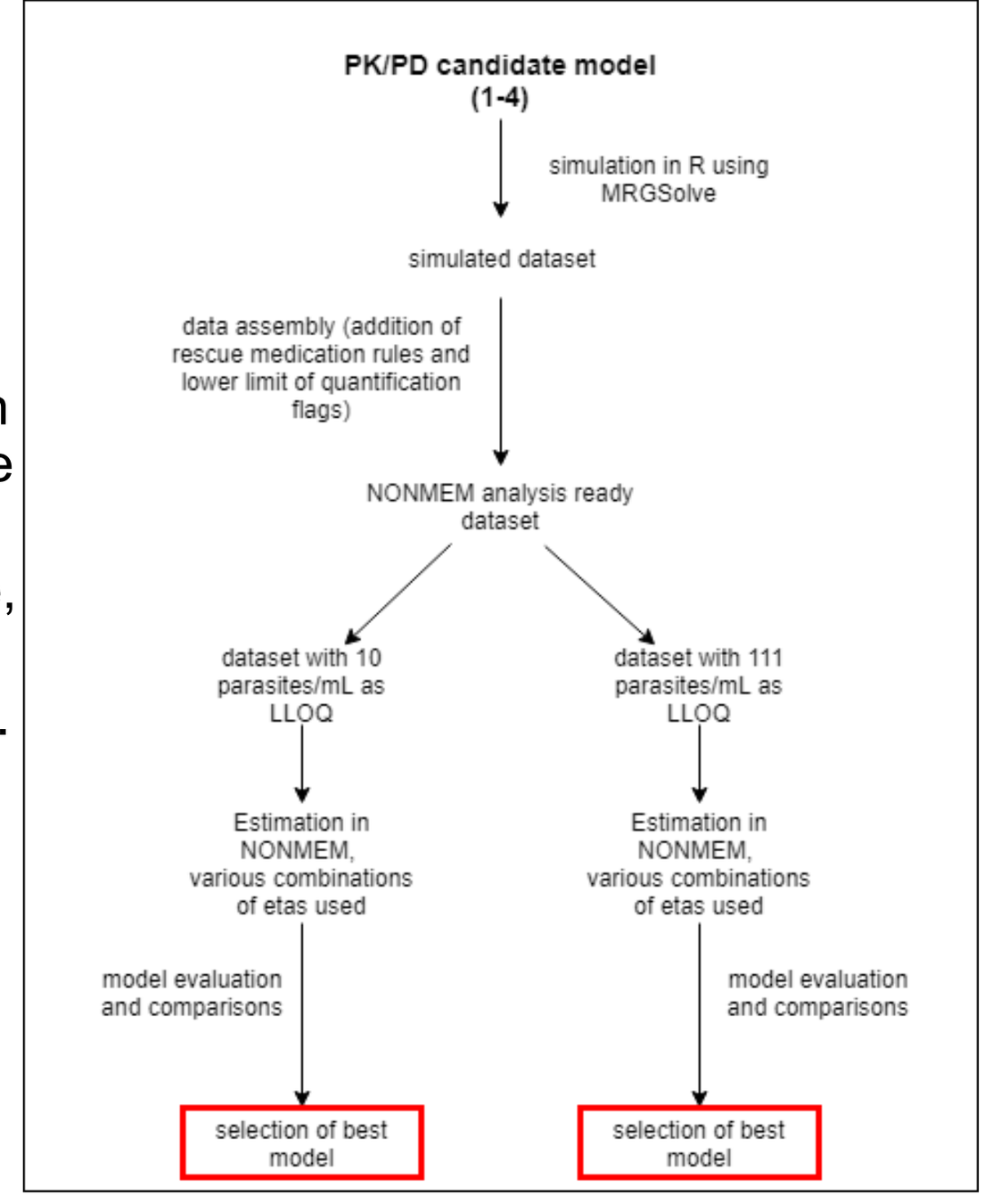

PK/PD candidate model
(1-4)
simulation in R using MRGSolve
simulated dataset
data assembly (addition of rescue medication rules and lower limit of quantification flags)
NONMEM analysis ready dataset
dataset with 10 parasites/mL as LLOQ
dataset with 111 parasites/mL as LLOQ
Estimation in NONMEM, various combinations of etas used
Estimation in NONMEM, various combinations of etas used
model evaluation and comparisons
model evaluation and comparisons
selection of best model
selection of best model

Figure 2. Workflow of Simulation-Estimation for IBSM Data Model Identifiability

- For each PD model evaluated, 2 sets of estimations occurred; 1 set where the LLOQ was 10 parasites/mL and 1 set with the LLOQ as 111 parasites/mL. This resulted in 2 estimation sets per candidate model.
- NONMEM estimation: Laplacian method and the M3 method for samples below the LLOQ.
- For each candidate model, various combinations of interindividual variability (IIV) in parameters were tested, as shown in Fig. 2. These combinations of IIV produce the number of models evaluate for a design.
- Evaluation of each model included a consideration of the minimum value of the objective function, successful covariance, parameter estimation (within 10% of the true value), and precision of parameter estimates (%RSE < 5).
- For models with successful covariance, goodness-of-fit plots were evaluated.
- From these criteria, each PD model was evaluated and the best model using each LLOQ value was selected (red boxes in Fig. 2).

RESULTS

Each of the 4 candidate models were able to fit the data, with varying degrees of success. Table 2 shows the number of models (varying by IIV structure) that had a successful covariance for each candidate model structure.

Table 2. Number of Models With Successful Covariance Step Completion, Stratified by the Lower Limit of Quantitation Value Used in Estimation

	Traditional		IDR		Gompertz		Gordi	
LLOQ value used in estimation (parasites/mL)	10	111	10	111	10	111	10	111
Number of models[a] with successful covariance	5/7	5/7	1/8	1/8	2/9	4/9	9/12	9/12

[a] Within a model type, the models differed in the number and location of random effects terms.

Traditional Model

- For both LLOQ datasets
  - Successful covariance: 5 of the 7 models.
  - Reasonable parameter estimates and precision in 2 of the 5 successful covariance models.
  - LLOQ = 10: the model with IIV on the inoculum value and K_d was selected as the best model.
    - PD parameter estimates and their CIs close to the true values of the simulations.
  - LLOQ = 111: the model with IIV on all parameters was selected as the best model.
    - PD parameter estimates and their CIs were very close to the true values.
- The model for the dataset with LLOQ = 111 parasites/mL yielded more accurate parameter estimates for Knet and K_d, as compared to the true values of the data.
- The model for the dataset with LLOQ = 10 parasites/mL yielded a better estimate of EC_{50}.

Indirect Response Component Model

- For both LLOQ datasets
  - Successful covariance: 1 of the 8 models.
  - Both models had high %RSE on EC_{50}.
- Dataset with LLOQ = 10: model with IIV on all parameters was successful; good estimates of K_g, K_d, and E_{max}.
- Dataset with LLOQ = 111: model with IIV on EC_{50}, E_{max}, and K_d was successful; accurate estimate of EC_{50}.
- Though over-parameterized, this model suggests a basis for discussion of separation of natural killing versus drug effect.

Gompertz Model

- Dataset with LLOQ = 10
  - Successful covariance: 2 of the 9 models.
  - Model with IIV on both EC_{50} and K_d had a good estimate of K_g, Ψ, and K_d and was chosen as the final model from this group.
- Dataset with LLOQ = 111 parasites/mL
  - Successful covariance: 4 of the 8 models.
  - Model with IIV on the inoculum, EC_{50} and K_d was the best model, but IIV EC_{50} estimate was (1310 %CV). Other fixed effect PD parameters were within 10% of the true value from the simulation.

Gordi Model

- Dataset with LLOQ = 10 parasites/mL
  - Successful covariance: 9 of the 12 models.
  - The model with IIV on Kinj, MTT, and the inoculum value had parameter estimates closest to the true values and was reasonably well estimated.
- Dataset with LLOQ = 111 parasites/mL
  - Successful covariance: 9 of the 12 models.
  - The model with IIV on Kinj, MTT, and the inoculum value was selected as the best model.
- A comparison between the 2 estimation sets showed the estimation, which used an LLOQ of 10 parasites/mL, yielded parameter estimates of MTT and K_d closer to the true values.

SUMMARY and NEXT STEPS

- A method to evaluate the identifiability of PD models used to characterize IBSM data was tested.
- The Gordi and the traditional model were the more identifiable models. The modified traditional models (that is, the IDR model and the Gompertz model) were not identifiable, but serve for discussion of modeling approaches.
- The results of the Gordi model simulation-estimation demonstrate that the VIS data can support the identifiability of a semi-mechanistic model.
- The traditional model is often used because of relative simplicity and the inclusion of a familiar potency (EC_{50}) parameter which can be translated throughout phases of development and comparisons between agents.
- The cyclical nature of parasite growth in IBSM studies has been well documented in the literature and is observable across subjects in the IBSM study design due to the synchronous administration of the parasite challenge across subjects. Cyclical data simulated from a sine function could be fit with both a linear growth model and a sine wave function; to facilitate the understanding of the effect of the estimation of PD parameters from collapsing the sine wave growth to a linear function.
- Similar to the workflow used in this analysis (Fig. 1), subsequent analyses could simulate datasets and investigate the effect of the proportion of patients who recrudesce on model identifiability.
- The rules which are used to censor data after rescue medication administration can be altered such that varying protocol designs can be evaluated for the ability of the data to inform the models.
- More mechanistic models should be pursued as study designs evolve, as biomarkers for stages of parasite lifecycles are identified and made available, and as combination treatments are explored.

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